

Watson Lake Limno-corral Study: Phase I

FINAL

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Table of Contents

INTRODUCTION	4
MATERIALS AND METHODS	6
RESULTS	9
Nutrients	9
Physico-chemical.....	15
Biological	19
Relationships.....	28
Discussion.....	32
Figure 1. Limno-corral Sketches.....	5
Figure 2. Map of Limno-corral Placed in Watson Lake.....	6
Figure 4. One-way Analysis of Nitrogen Levels by Treatment	9
Figure 5. Levels of Ortho-phosphate and Total P by Treatment.....	10
Figure 6. Mean Sum of all Nutrient Levels by Treatment	10
Figure 7. Mean Sum of Total Nutrient Levels for all Depths by Site	11
Figure 8. Nitrogen Levels by Pooled Site.....	11
Figure 9. Phosphorous Levels by Pooled Site.....	12
Figure 10. Nitrogen Levels by Depth for Limno-A.....	12
Figure 11. Levels of Orthophosphate and Total P by Depth at Site A.....	13
Figure 12. Levels of Total and Orthophosphate with Depth at Limno-B	13
Figure 13. Nitrogen Levels with Depth at Site Limno-B	14
Figure 14. Temperatures Profiles by Depth and Site (Depth in Meters)	15
Figure 15. Dissolved Oxygen Levels by Depth and Site (Depth in Meters)	16
Figure 16. pH by Depth and Site (Depth in Meters).....	17
Figure 17. Secchi Depth by Treatment.....	18
Figure 18. Turbidity Levels by Treatment	18
Figure 19. Chlorophyll A Levels by Treatment	19
Figure 20. Chlorophyll A by Depth and Site (Depth in Meters)	20
Figure 21. C:P Ratio by Treatment	21
Figure 22. C:P Ratio by Depth and Site (Depth in Meters).....	22
Table 1. Algae Results	22
Figure 23. Units/mL vs. Division by Treatment.....	23

Figure 24. Ln(Biovolume) vs. Division by Treatment	24
Figure 25. Units/mL vs. Genus by Treatment and Pooled Sites.....	25
Figure 26. Biovolume Percent Total vs. Genus by Treatment and Pooled Sites.....	26
Figure 27. Number M^3 vs. Order by Treatment and Pooled Sites.....	27
Figure 28. Number M^3 vs. Order by Treatment and Pooled Sites	28
Picture 1. <i>Gloecystis</i> sp. found within Watson Lake limno-corrals 100 X.....	34
Picture 2. <i>Spirogyra</i> sp. found within Watson Lake limno-corrals. 150 X.....	35
Picture 3. <i>Chlamydomonas</i> sp. found within Watson Lake limno-corrals. 200 X.....	35
Picture 4. Limno-corrals loaded on pontoon boat for deployment.....	36
Picture 5. Limno-corrals deployed within Watson Lake.....	36
Picture 6. Outside of limno-corrals.	37
Picture 7. Collecting zooplankton from limno-corrals.....	37

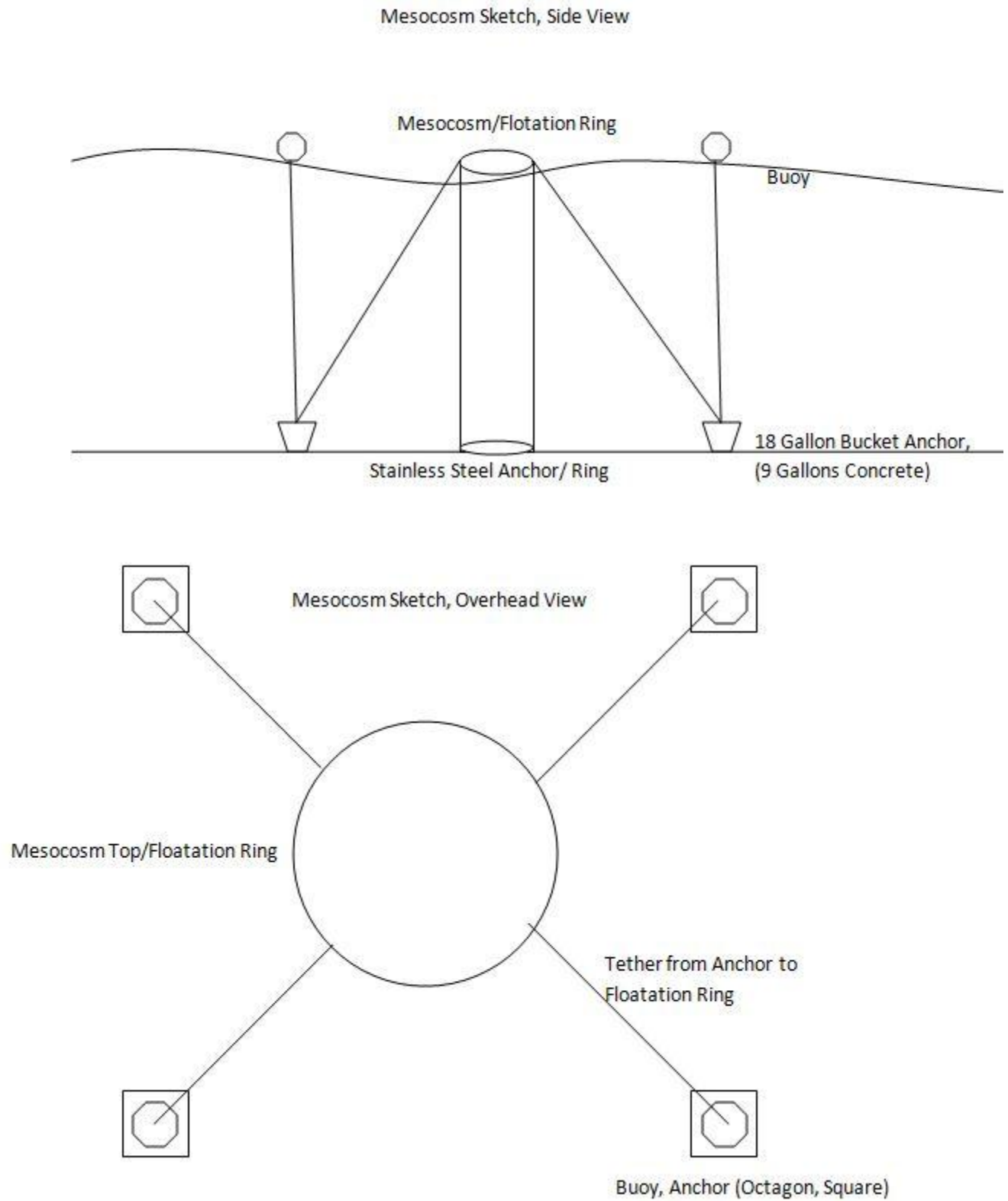
INTRODUCTION

Subtle changes in lake or reservoir trophic status are often difficult to quantify. Most changes occur over years if not decades and are insidious. Some lakes progress in a linear fashion toward increasing productivity and some do not. Trying to quantify those changes, when they occurred and the biological response is extraordinarily difficult. It's been almost 60 years since G.W. Hutchinson published the Paradox of the Plankton which postulates that competitive exclusion amongst algal assemblages seems to be lacking given that they live in a "homogenous" environment. The answer, of course, is that diversity among algal species is maintained because they do not live in a homogenous environment. Although the dynamic nature of an aqueous environment is now generally understood, we still know little about specific environmental requirements for any given alga to grow and survive. We know even less about interactions among algal species yet we are often asked to make determinations and predictions about how they will respond to a given set of environmental circumstances either as they exist now or in the future. All of these determinations and predictions about algal assemblage changes due to changing environments need to be scrutinized because we cannot yet quantify the myriad of very subtle and highly dynamic environmental conditions that undoubtedly are of importance to any algal assemblage. Environmental models can sometimes make fairly accurate determinations on a gross scale but often fail at determining the subtle and highly dynamic environments in which algal assemblages reside.

Controlled conditions in a laboratory setting makes determining specific requirements needed for a given alga to grow and survive easier to ascertain, however, such studies often lack environmental significance because exact environmental conditions can never be fully reproduced in such a controlled setting. Disturbance and chaos is an important environmental variable that can almost never be reproduced in the laboratory because such events are highly stochastic. At the other end of the scale, it's very difficult to determine significance in field-based studies due to a lack of control and replication. Both approaches have merit and also significant drawbacks. *In situ* studies that offer a relatively high degree of environmental significance but also allowed for some control and replication may enable an enhanced understanding of algal assemblage ecology over either a strict laboratory- or field-based approach alone.

The use of mesocosms called "limno-corrals" have been extensively used in several lake studies to determine assemblage and trophic changes due to a variety of treatments (Pilati & Wurtsbaugh 2003, Patterson et. al. 1997, Stewart 1999, Levine & Schindler 1999, Klug 2003, Nydick et. al. 2004, Padisak 1992). Limno-corrals allow some control and replication yet being *in situ* very closely mimic ambient lake conditions such as photo-period, temperature, light intensity, and native aquatic organisms. Limno-corrals are transparent tubes made of various inert materials (Fig. 1) that extend from above the water's surface and are fitted over lake sediment. Thus, they contain a walled-off column of lake water wherein treatments and manipulations can occur. They are ideal for determining native organism's response to a variety of treatments including fertilization, nutrient limitation, trophic structure and energy exchange, etc.

Figure 1. Limno-corral Sketches



MATERIALS AND METHODS

Four limno-corrals were deployed at two locations within Watson Lake Arizona on 8/16/11 (Fig. 2). Two limno-corrals were deployed in the lacustrine area near the dam and two in a transitional area between the dam and in-coming Granite Creek.

Figure 2. Map of Limno-corrals Placed in Watson Lake



The general experimental design was to collect baseline information, then fertilize with nitrogen and phosphorous, and then add aluminum sulfate (alum) to make P limiting (two treatments). We would then observe physico-chemical, chemical, and especially biotic (primarily phytoplankton) responses to fertilization and nutrient limitation. The basic timeline for each treatment is given below:

- 8/17/11 – 8/31/11: Baseline
- 8/31/11 – 9/14/11: Fertilization
- 9/28/11 – 10/12/11: Alum addition

We used a Hydrolab ® Surveyor 4a datasonde and display to collect water temperature, pH, dissolved oxygen (% saturation and mg/L), specific conductivity, and oxidation-reduction potential (ORP), every 1.0 meter throughout the water column in each limno-corral.

Water chemistries and biological samples were collected by lowering tygon tubing to a specified depth and water was pumped into collection containers using a peristaltic pump.

Generally, samples were collected just beneath the water's surface, at a mid-point in the water column, and ~ 1.0 meter above lake sediment. Water chemistries were submitted to Xenco Laboratories and biological samples were brought back to the University of Arizona Environmental Research Laboratory (ERL) in Tucson, Arizona. Turbidity levels were determined in the field (surface, middle, and bottom) using a Hach® turbidity meter. Water chemistries and biological samples included:

Chemistry

Ammonia
Nitrate+Nitrite
Total Kjeldahl Nitrogen
Ortho-phosphate
Total Phosphorous
Total Alkalinity
Total Dissolved Solids
Total Suspended Solids
Chloride
Fluoride
Sulfate

Biological

Chlorophyll *a*
Algae Count and Identification (#'s/mL and biovolume)
Zooplankton

Algae counts and identification were performed using a Sedgewick-Rafter counting chamber on an Olympus BH2 phase-contrast microscope. Counts were natural unit counts and identified to Genus. Zooplankton were collected with zooplankton nets as a vertical pull from the bottom to the water's surface of each limno-coral using two mesh sizes; 80 and 243 µm. Algae and zooplankton samples were preserved with a 2-3% (total concentration) formalin or glutaraldehyde solution.

Limno-coralls were purchased from Aquatic Research Instruments and consisted of a polyethylene fabric that was 1.0 meter in diameter (Fig. 3). Light transparency was ~ 85% of ambient levels. Floats were fabricated (plastic tubes filled with Styrofoam) so that the top of each limno-coral was above the surface of the water. Weights (concrete blocks) and stainless steel hoops were used to secure the bottom of each limno-coral over lake sediment. Four additional anchors (12 gallons of concrete each) were dropped to the bottom of the lake and the top of each limno-coral was secured to each anchor at equidistant points around the circular opening at the top. Hoops constructed of PVC were inserted into openings every 10 feet in each limno-coral to aid in them staying open. Limno-coralls were installed in the lake on 8/16/12. Baseline measurements were obtained on 8/17/12.

Commercial N and P used in the aquarium trade (Flourish Phosphorous TM and Flourish Nitrogen TM) were purchased and added to each mesocosm during the fertilization treatment. Enough was added to each limno-coral (total volume was depth-dependent) to attempt an approximate concentration of 5.0 mg/L of total N and 0.75 mg/L of total P.

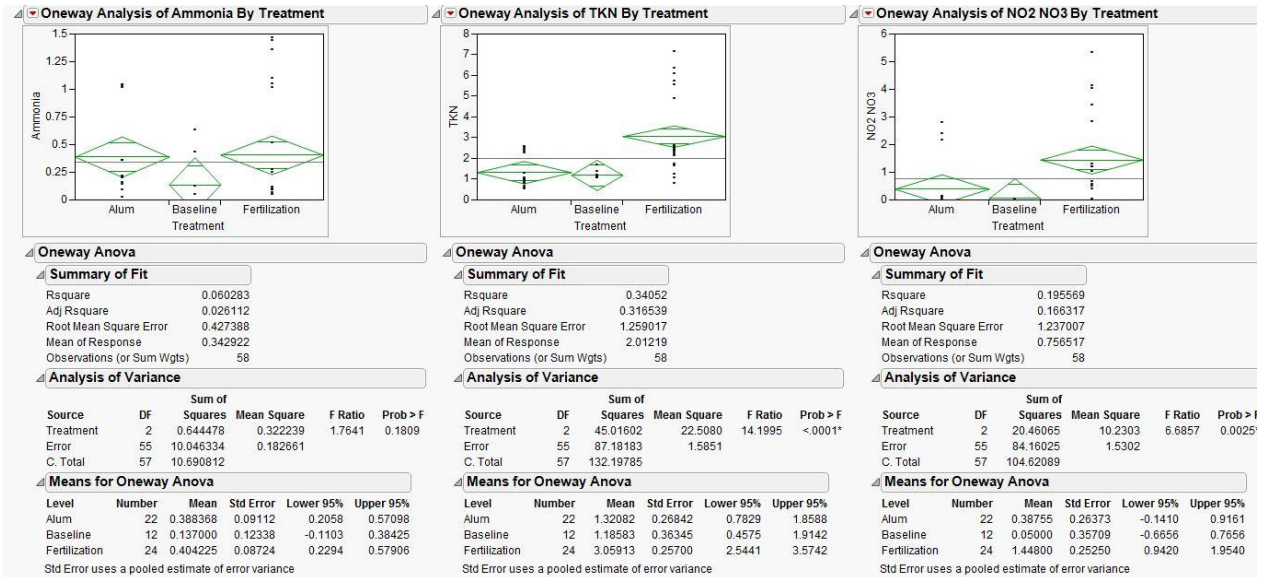
Granular, un-buffered aluminum sulfate was made into a slurry using lake water and added to each limno-coral via a peristaltic pump during the P removal (or “alum”) treatment. Alum dose was calculated using the last total P results from each mesocosm and based upon the volume of each limno-coral. Alum dosing attempted to re-establish total P levels found during the baseline measurements.

RESULTS

Nutrients

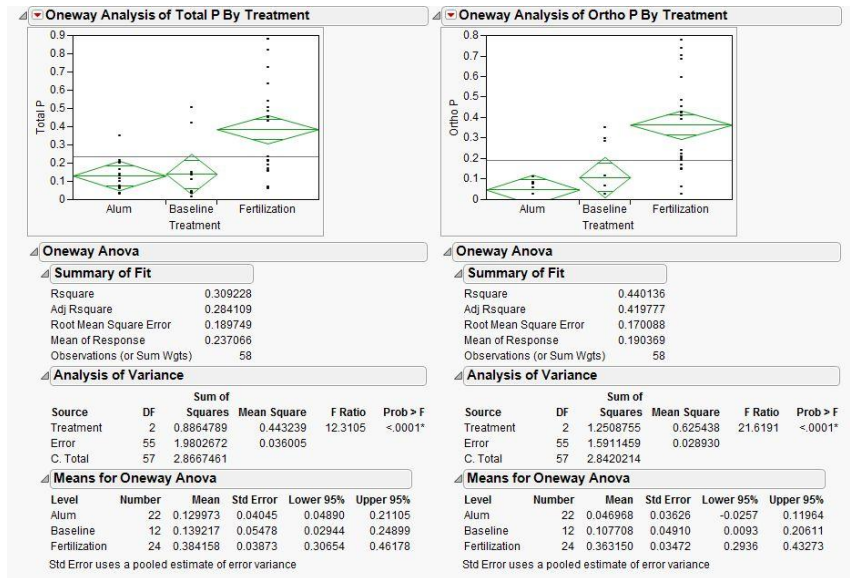
As a mean of all sites, levels of nitrate+nitrite and TKN were significantly higher during the fertilization treatment than the alum addition or baseline (Fig. 4). Levels of ammonia were not significantly lowered with alum and were significantly lower at baseline conditions.

Figure 4. One-way Analysis of Nitrogen Levels by Treatment



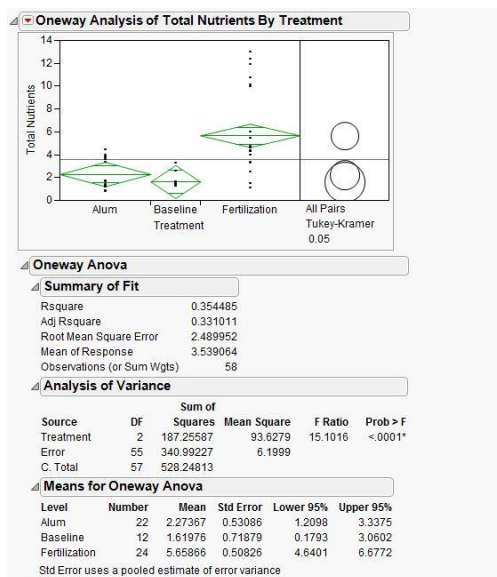
As a mean of all sites, levels of both ortho-phosphate and total P were significantly greater during the fertilization treatment than the alum or baseline conditions (Fig. 5). Levels of ortho-phosphate were significantly lower during the alum treatment than either the baseline or fertilization condition. Levels of total P were almost identical between the alum and baseline condition.

Figure 5. Levels of Ortho-phosphate and Total P by Treatment



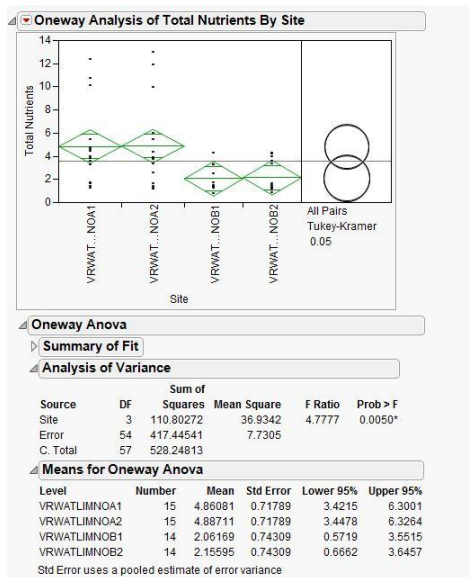
As the mean sum of all nutrients and all sites by treatment, levels were much greater during fertilization than the other treatments which were not significantly different (Fig. 6). Levels of total nutrients were not significantly different between the alum treatment and baseline condition.

Figure 6. Mean Sum of all Nutrient Levels by Treatment



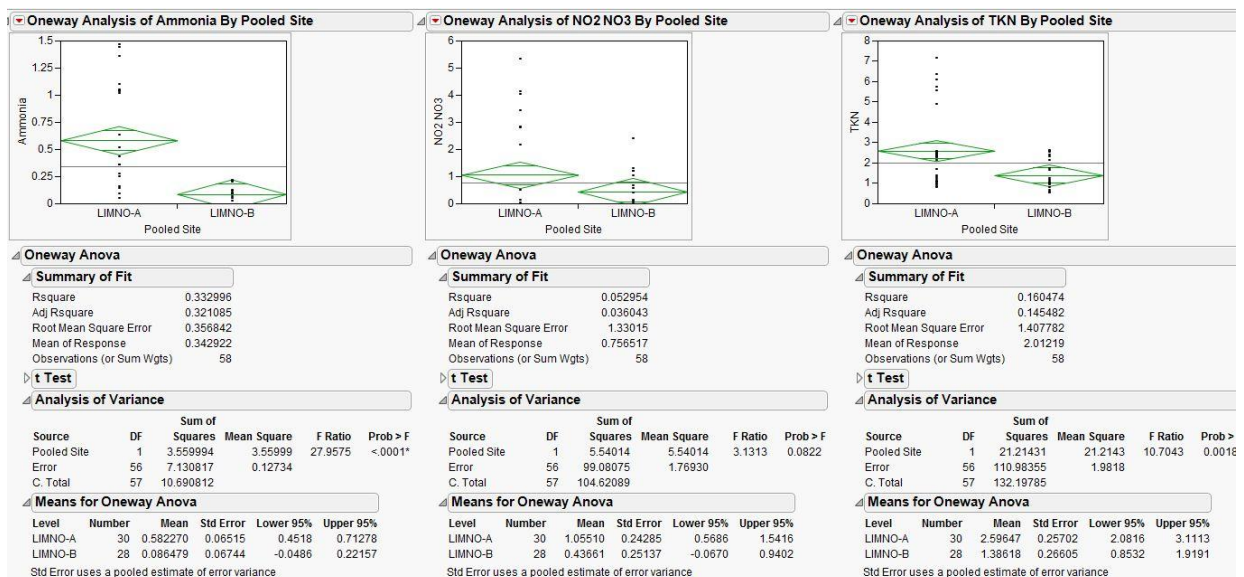
There were significantly higher levels of total nutrients at Site A than B (Fig. 7), however, there was no statistical difference in total nutrient levels between the replicates at either site. From here on, replicates will be pooled for analyses.

Figure 7. Mean Sum of Total Nutrient Levels for all Depths by Site



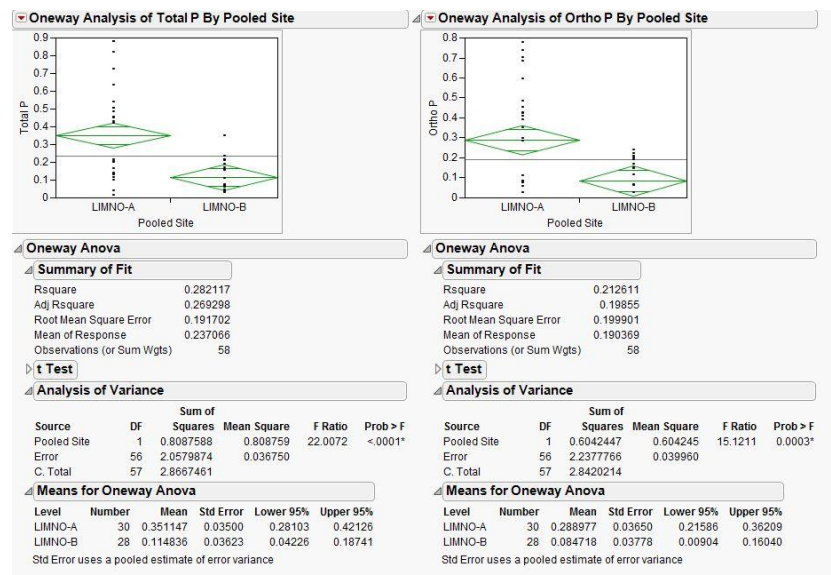
By pooling the replicates at each site, Site A showed significantly higher levels of all forms of nitrogen than site B (Fig. 8). The greatest difference existed in levels of ammonia.

Figure 8. Nitrogen Levels by Pooled Site



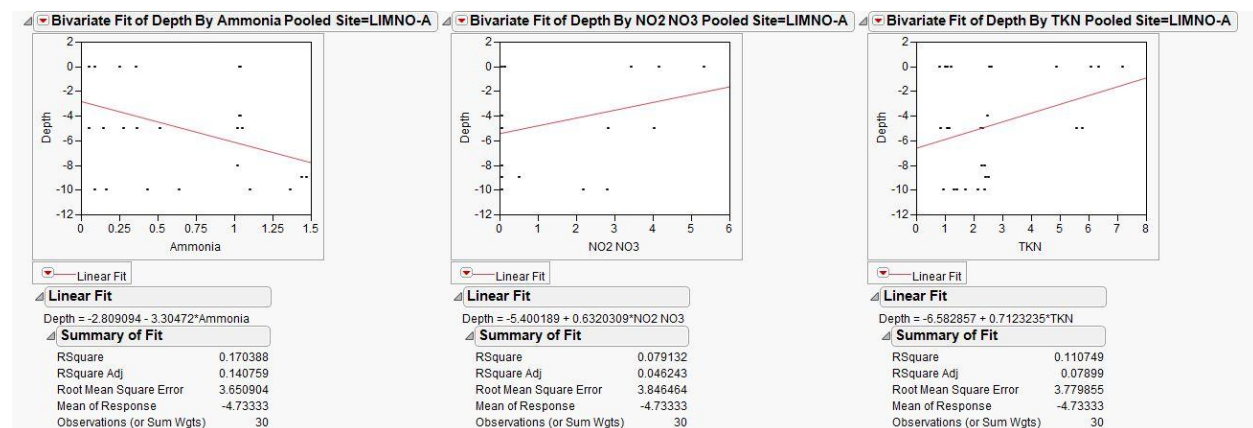
Limno site A also had significantly higher levels of total P and orthophosphate than did Limno site B (Fig. 9).

Figure 9. Phosphorous Levels by Pooled Site



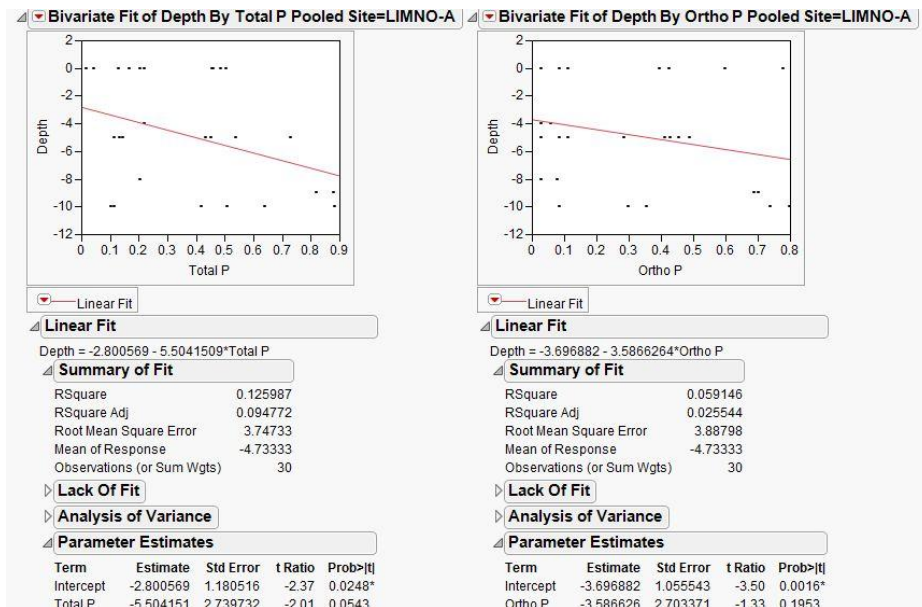
Pooled site A results showed a general increase in ammonia with depth but a decrease in TKN and nitrate+nitrite (Fig. 10).

Figure 10. Nitrogen Levels by Depth for Limno-A.



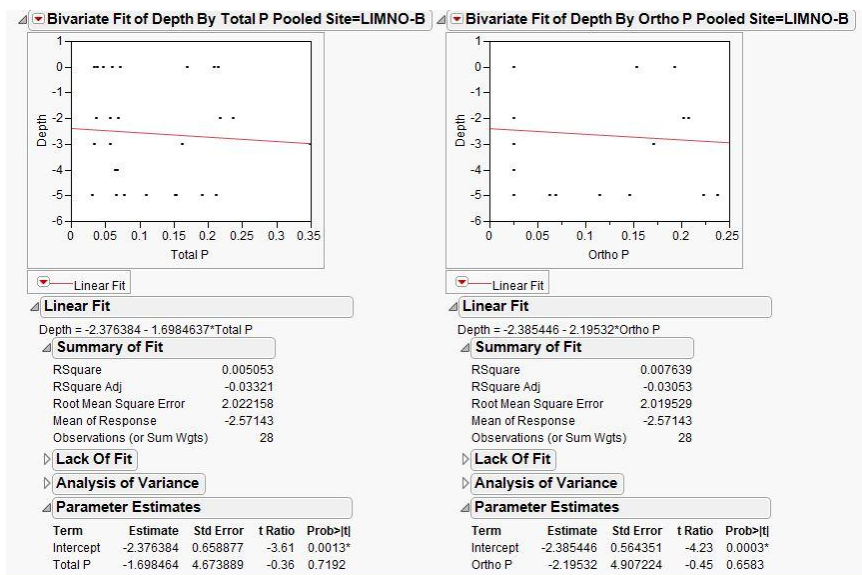
Levels of both orthophosphate and total P showed increases with depth at site A (Fig. 11).

Figure 11. Levels of Orthophosphate and Total P by Depth at Site A



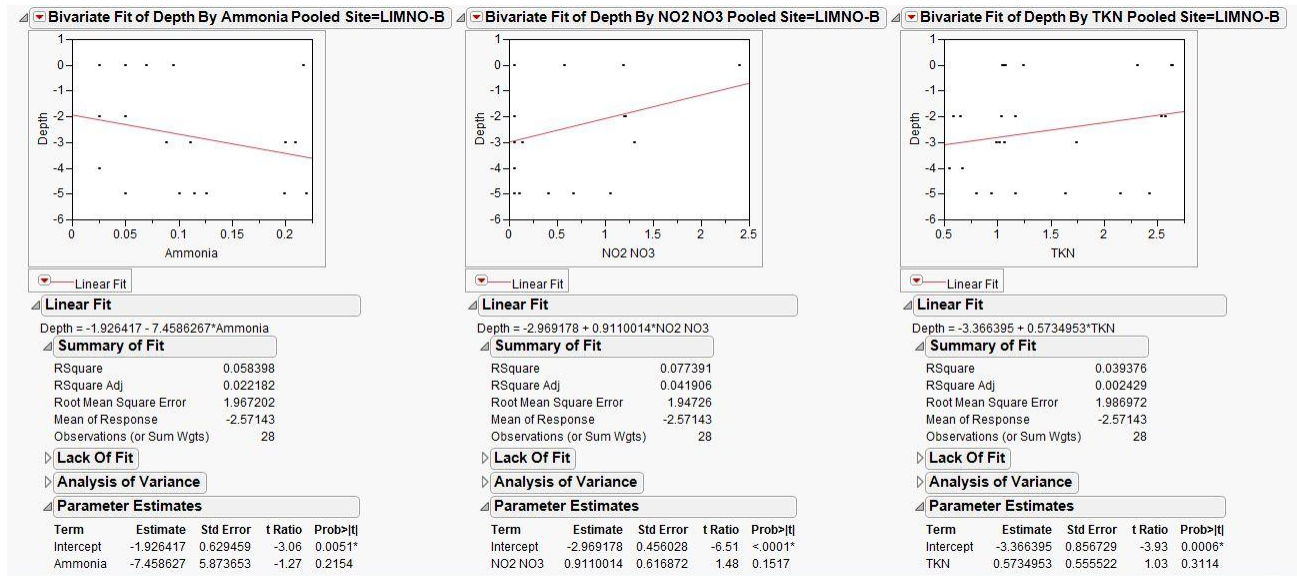
The effect of increased orthophosphate and total P with depth was not as noticeable as site B (Fig. 12).

Figure 12. Levels of Total and Orthophosphate with Depth at Limno-B



Interestingly, nitrogen levels at site Limno-B showed the opposite trend in nitrogen levels with depth as did site Limno-A (Fig. 13). Levels of ammonia decreased with depth but levels of nitrate + nitrite and TKN increased.

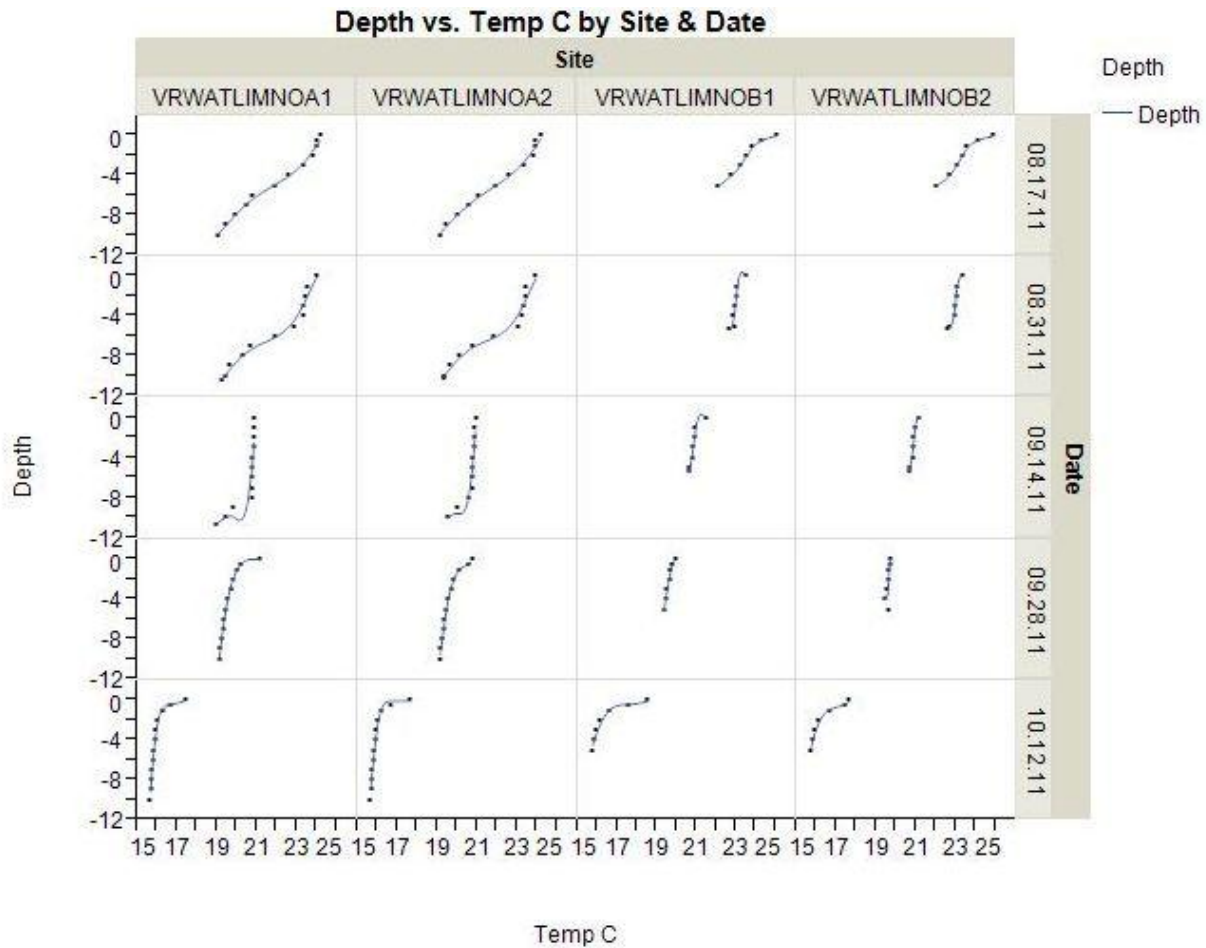
Figure 13. Nitrogen Levels with Depth at Site Limno-B



Physico-chemical

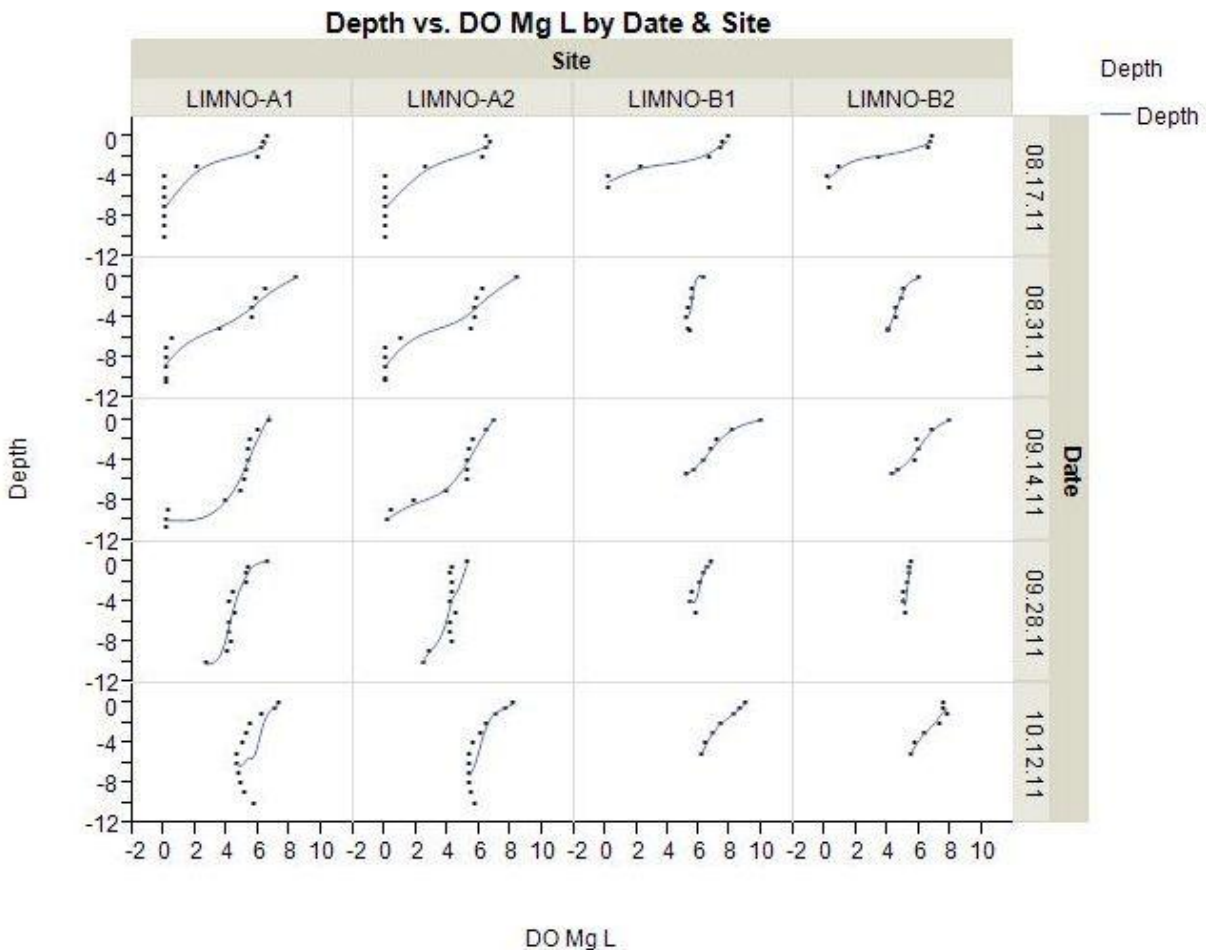
Water within Limno-A showed evidence of thermal stratification during the 8/17/12 and 8/31/12 data but then became more-or-less mixed for the remainder of the samplings (Fig. 14). Water within Limno-B showed far less thermal stratification than did Limno-A for all time periods (Fig. 14).

Figure 14. Temperatures Profiles by Depth and Site (Depth in Meters)



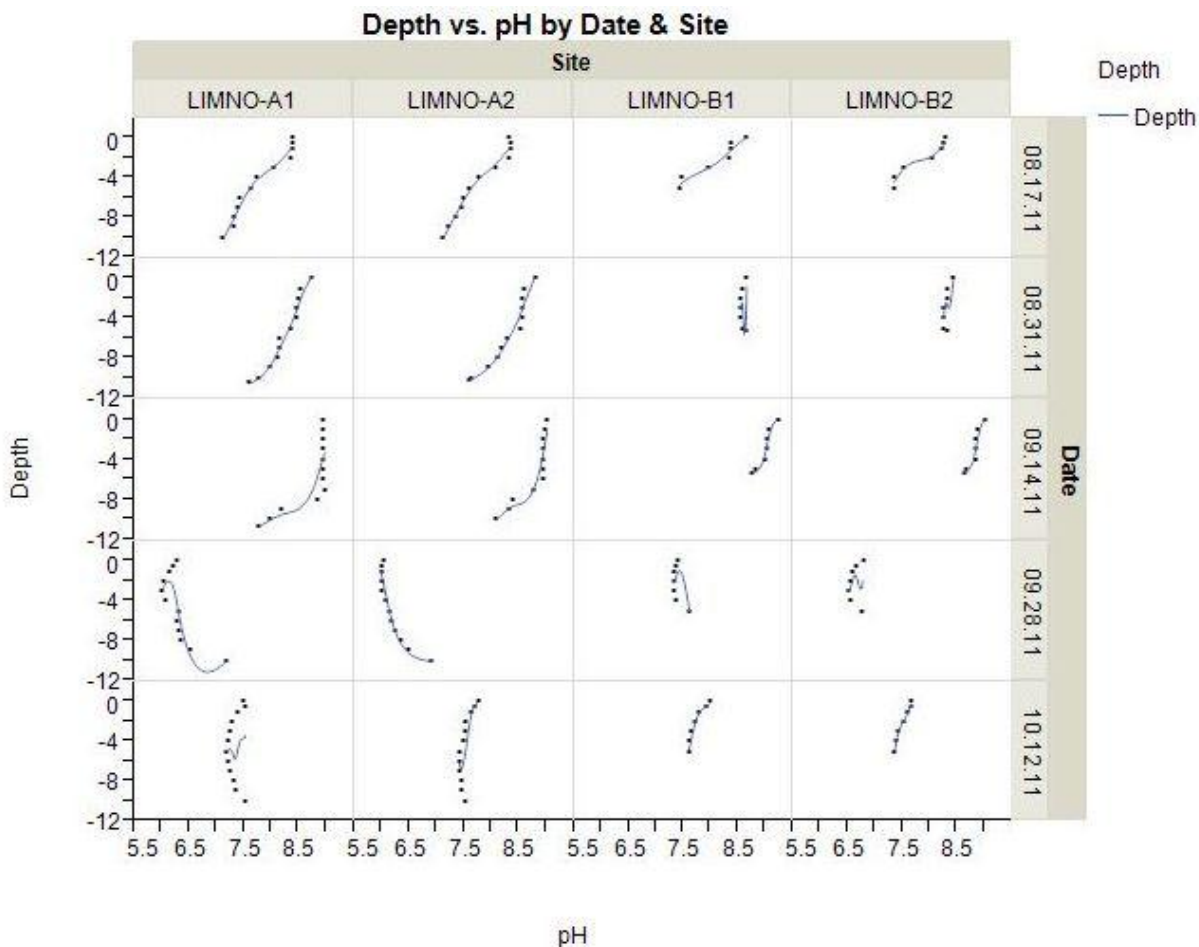
Levels of dissolved oxygen decreased with depth at both sites (Fig. 15). Site Limno-A showed anoxic conditions after 4-5 m during the 8/17 and 8/31/11 samplings (Fig. 15). Anoxia occurred at approximately 8 m during the 9/14/11 samplings with more-or-less mixed conditions upon subsequent samplings at this site. Site Limno-B exhibited anoxia after approximately 3 m only during the 8/17/11 sampling after which the water appeared to be relatively mixed.

Figure 15. Dissolved Oxygen Levels by Depth and Site (Depth in Meters)



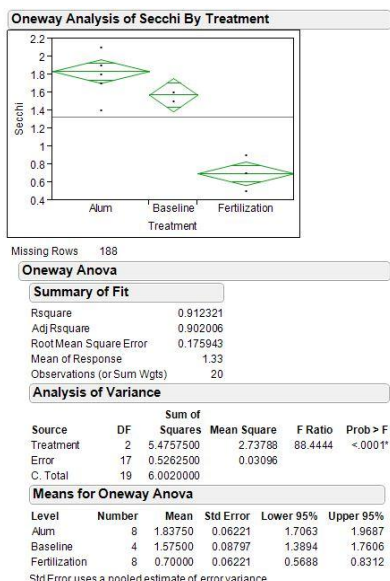
Levels of pH were generally higher at the surface for all sites (Fig. 16) except immediately following the alum treatment when levels were lower at the surface than at depth.

Figure 16. pH by Depth and Site (Depth in Meters)



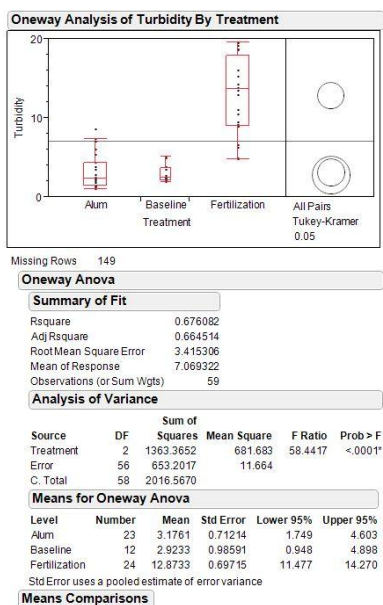
Secchi disk depth was significantly decreased during the fertilization treatment (Fig. 17). There was little to no difference in Secchi depth between the alum treatment and baseline condition.

Figure 17. Secchi Depth by Treatment



Levels of turbidity by treatment, as would be expected, were generally the inverse of those for Secchi disk depth with the fertilization treatment having the greatest levels of turbidity (Fig. 18). There was no significant difference between the baseline condition and alum treatment.

Figure 18. Turbidity Levels by Treatment



Biological

Despite significant changes in nutrient levels and water clarity between treatments, there was no significant difference in chlorophyll a levels by treatment (Fig. 19). Chlorophyll a levels by date, however, showed that levels were very low immediately following alum treatment; even lower than the baseline condition (Fig. 20). Highest levels were found during the latter parts of the fertilization and alum treatments (Fig. 20).

Figure 19. Chlorophyll A Levels by Treatment

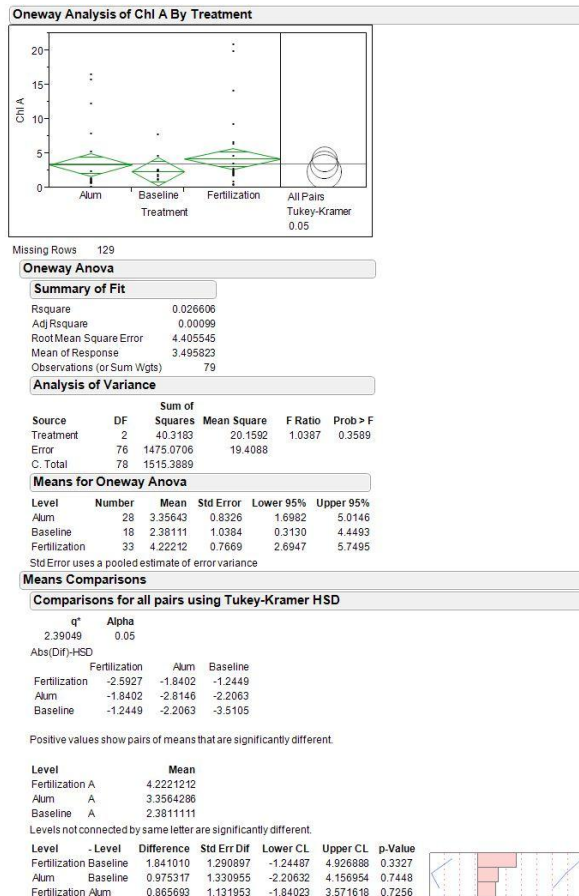
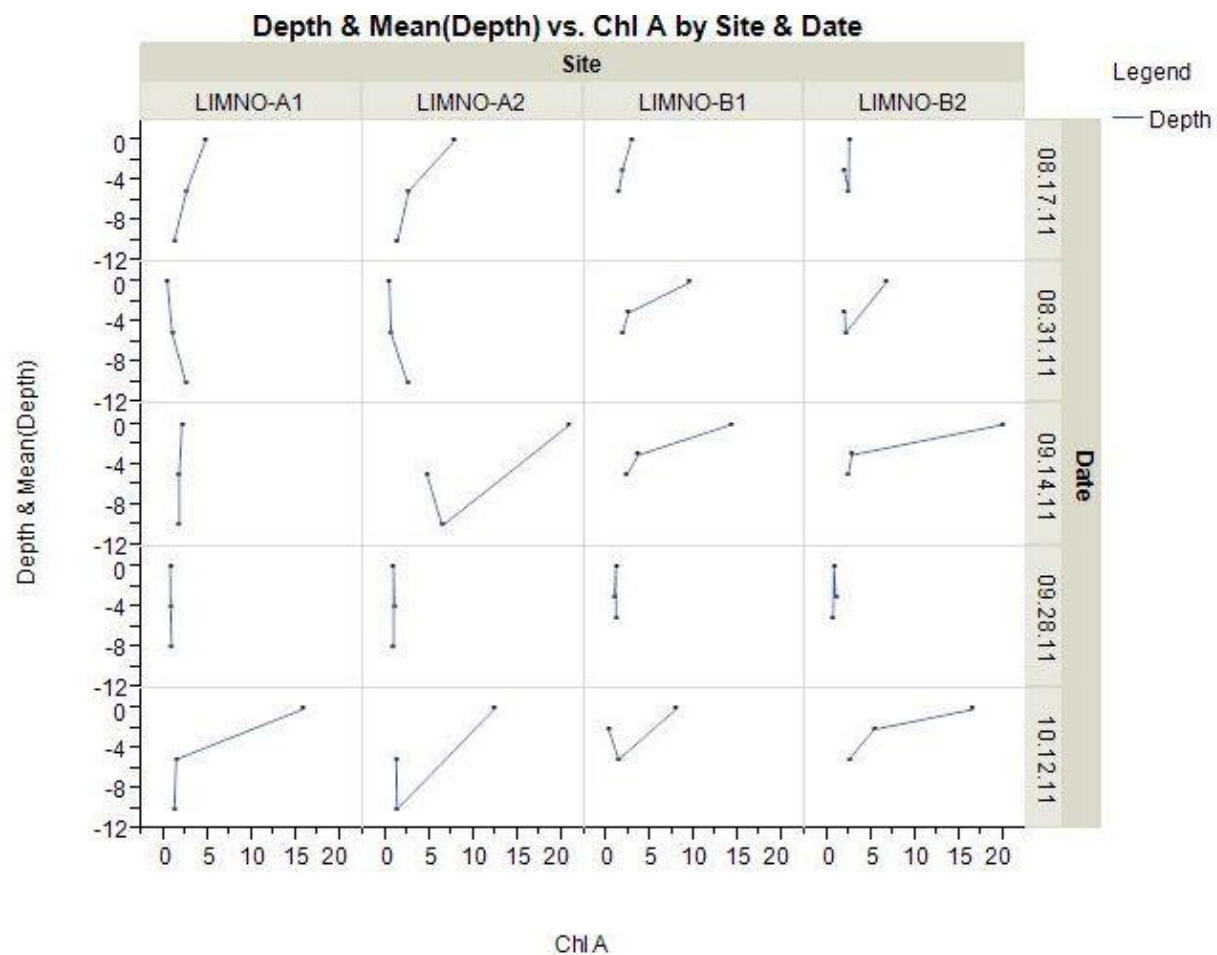


Figure 20. Chlorophyll A by Depth and Site (Depth in Meters)



The chlorophyll a:pheophytin ratio (a measure of cellular “health” since pheophytin is a degradation by-product of chlorophyll) indicates that algal cells were more degraded during the baseline condition and fertilization treatment than during the alum treatment (Fig. 21). By date, it appeared that the latter part of the alum treatment had the lowest C:P ratio, especially at site Limno-A (Fig. 22).

Figure 21. C:P Ratio by Treatment

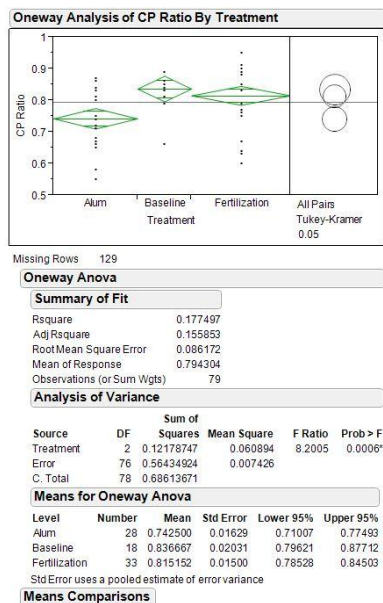
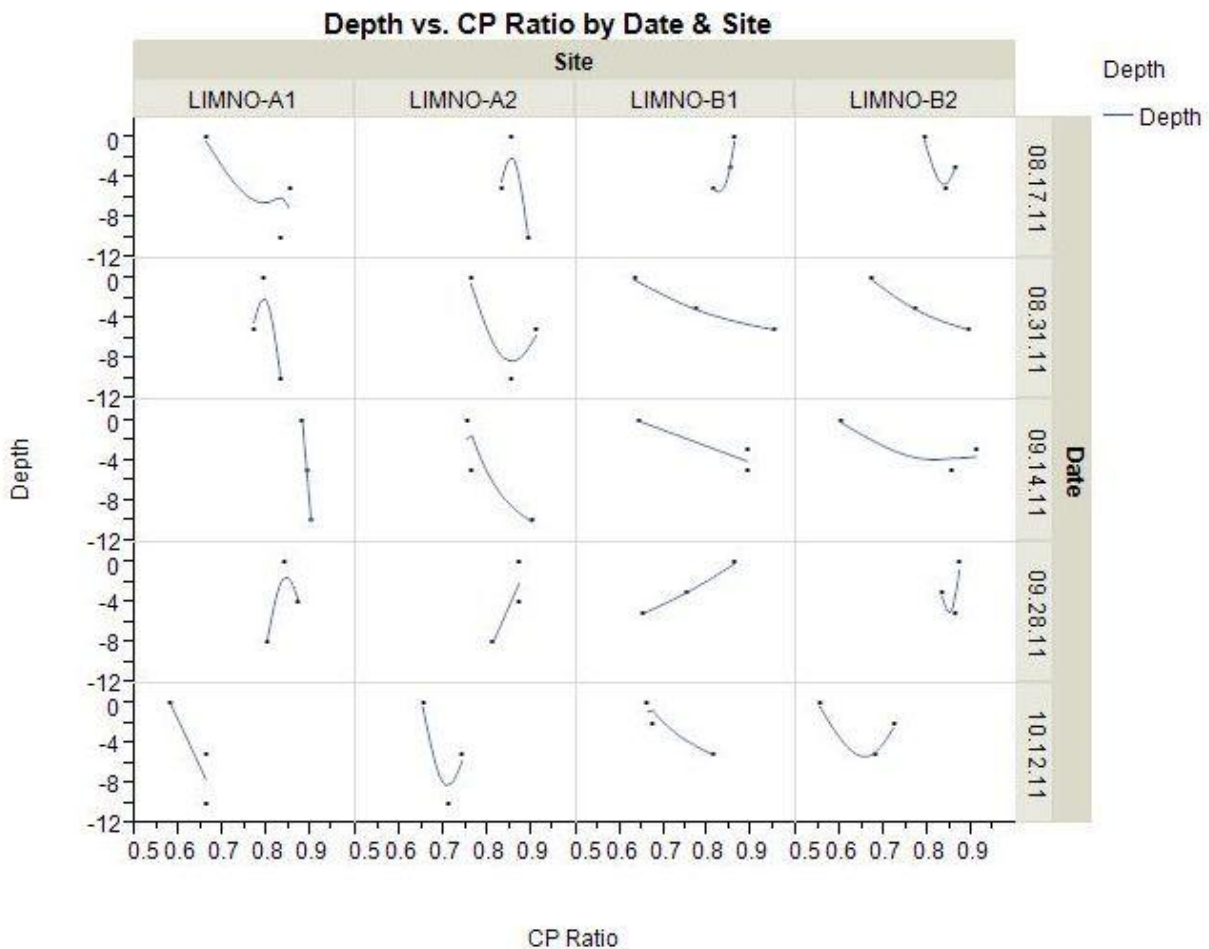


Figure 22. C:P Ratio by Depth and Site (Depth in Meters)



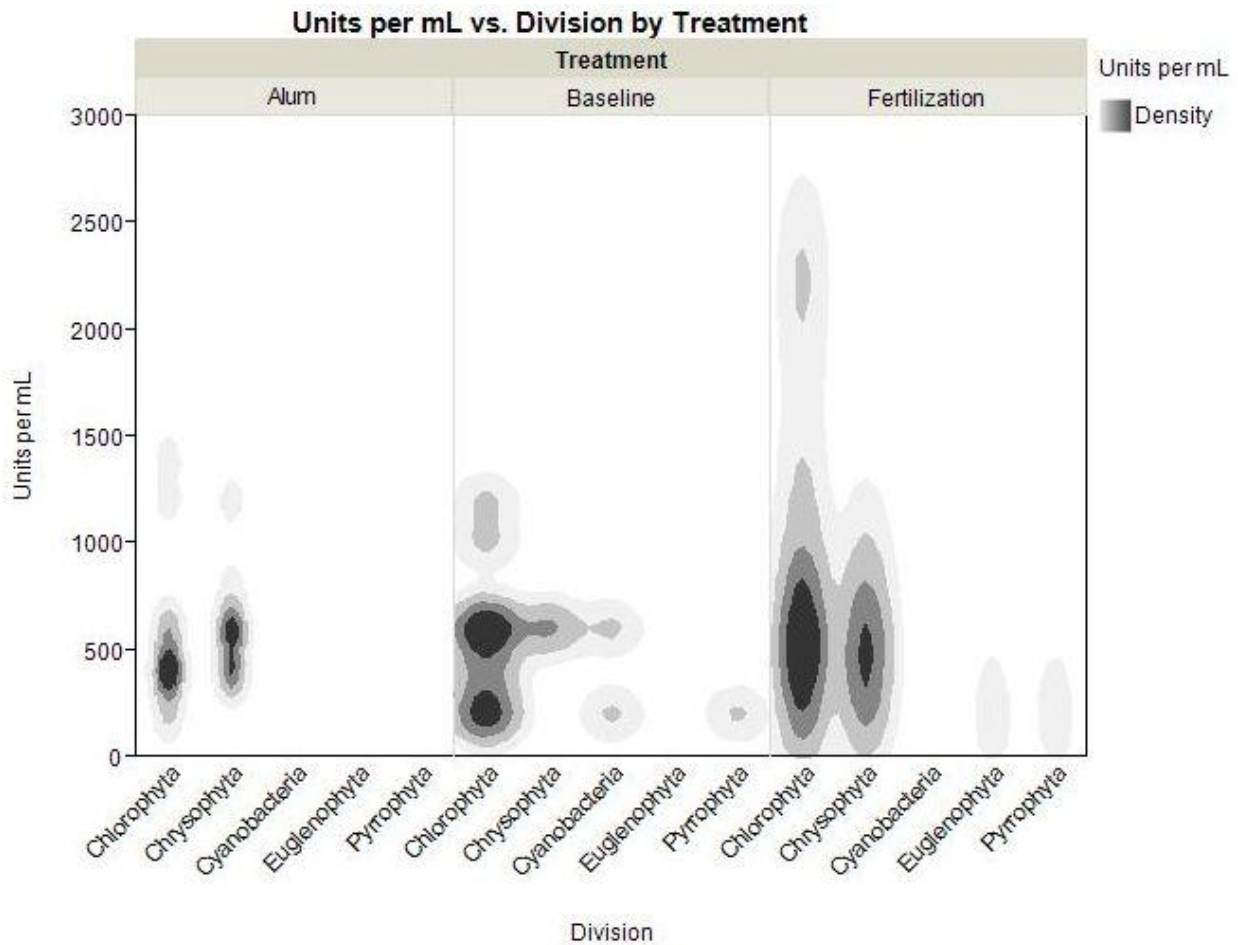
Overall, 10 species of algae were found during this project comprising 5 Divisions (Table 1). In terms of most prevalent, Chlorophytes dominated the phytoplankton with *Chlamydomonas* being the most frequently observed and *Spirogyra* having the greatest units/mL. The cyanobacter *Gloeotrichia* was found only twice but, by a large margin, had the largest biovolume.

Table 1. Algae Results

DIVISION	GENUS	FREQUENCY	Mean UNITS/m ²	Mean Biovolume (ln)
Chlorophyta	Chlamydomonas	19	715.79	5.95
Chlorophyta	Pediastrum	2	300.00	6.91
Chlorophyta	Scenedesmus	1	200.00	5.67
Chlorophyta	Spirogyra	12	1316.67	7.78
Chlorophyta	Staurastrum	5	267.10	6.75
Chlorophyta	Tetraselmis	10	540.00	6.13
Chrysophyta	Diatoma	25	560.00	5.80
Cyanobacteria	Gloeotrichia	2	400.00	9.77
Euglenophyta	Euglena	1	200.00	5.86
Pyrrophyta	Ceratium	2	200.00	7.21

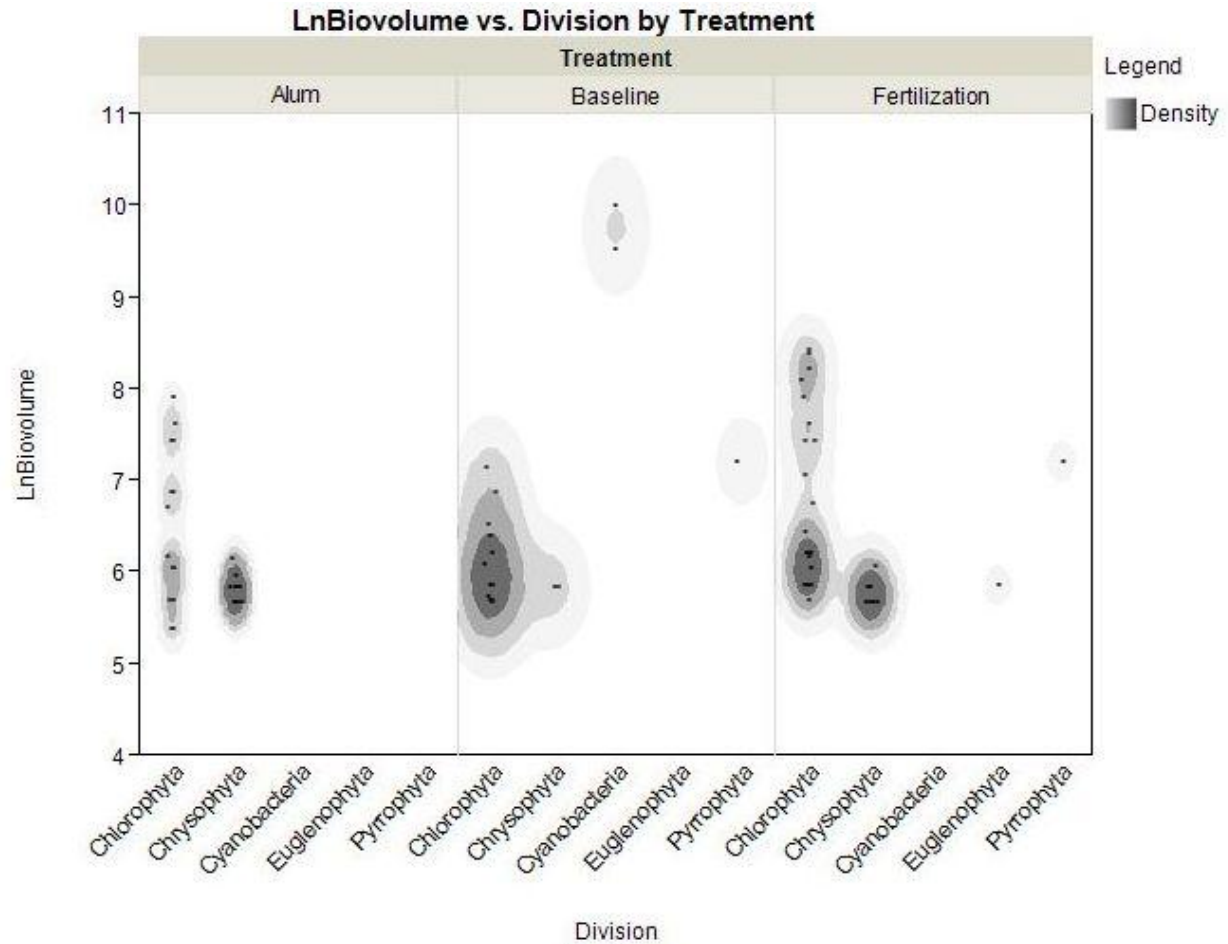
Algae counts on a unit/mL basis showed that chlorophytes and chrysophytes dominated the assemblage during all treatments and conditions (Fig. 23). Although there was no statistical difference between means for treatments, the highest individual counts were observed during the fertilization treatment.

Figure 23. Units/mL vs. Division by Treatment



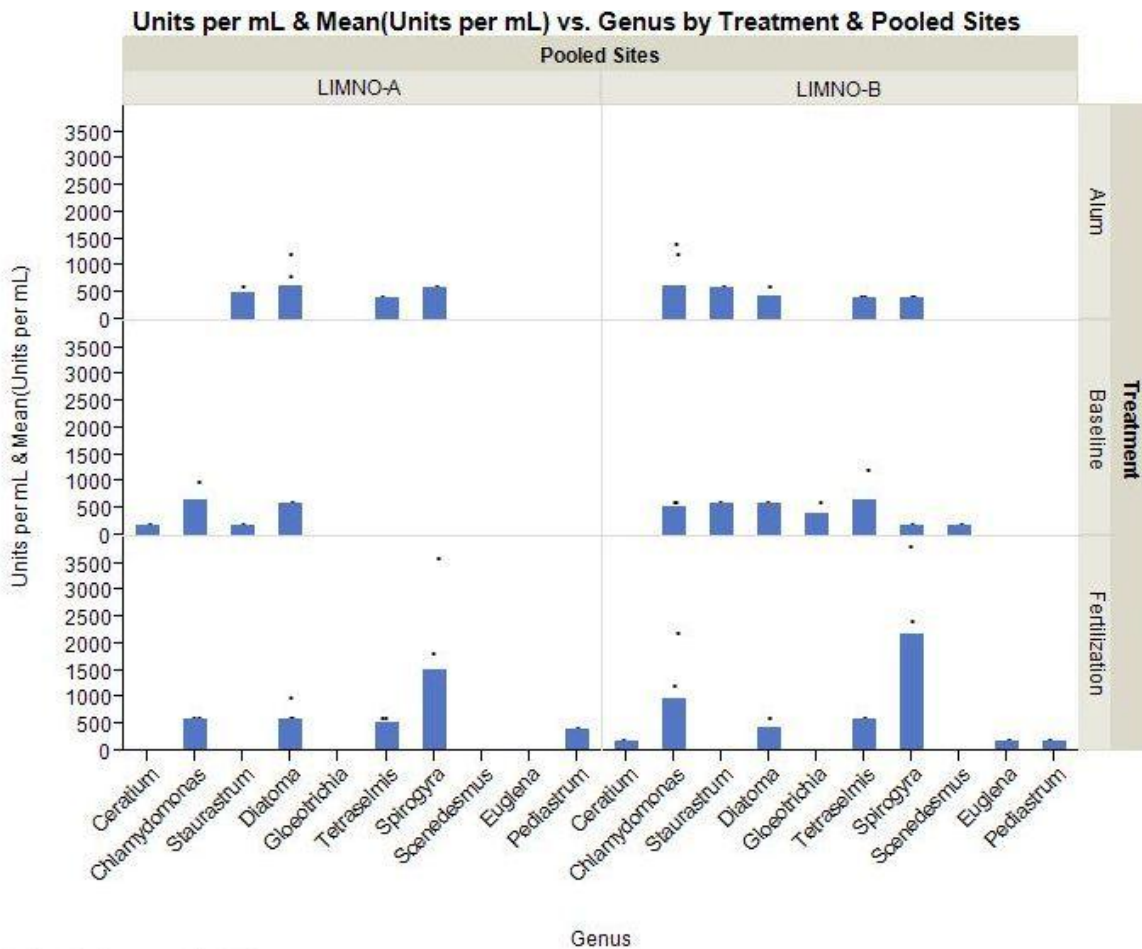
On a biovolumetric basis, there was no statistical difference between overall means based upon treatment or condition. On a Divisional basis, cyanobacteria found during the baseline condition had the highest biovolumetric numbers (Fig. 24).

Figure 24. Ln(Biovolume) vs. Division by Treatment



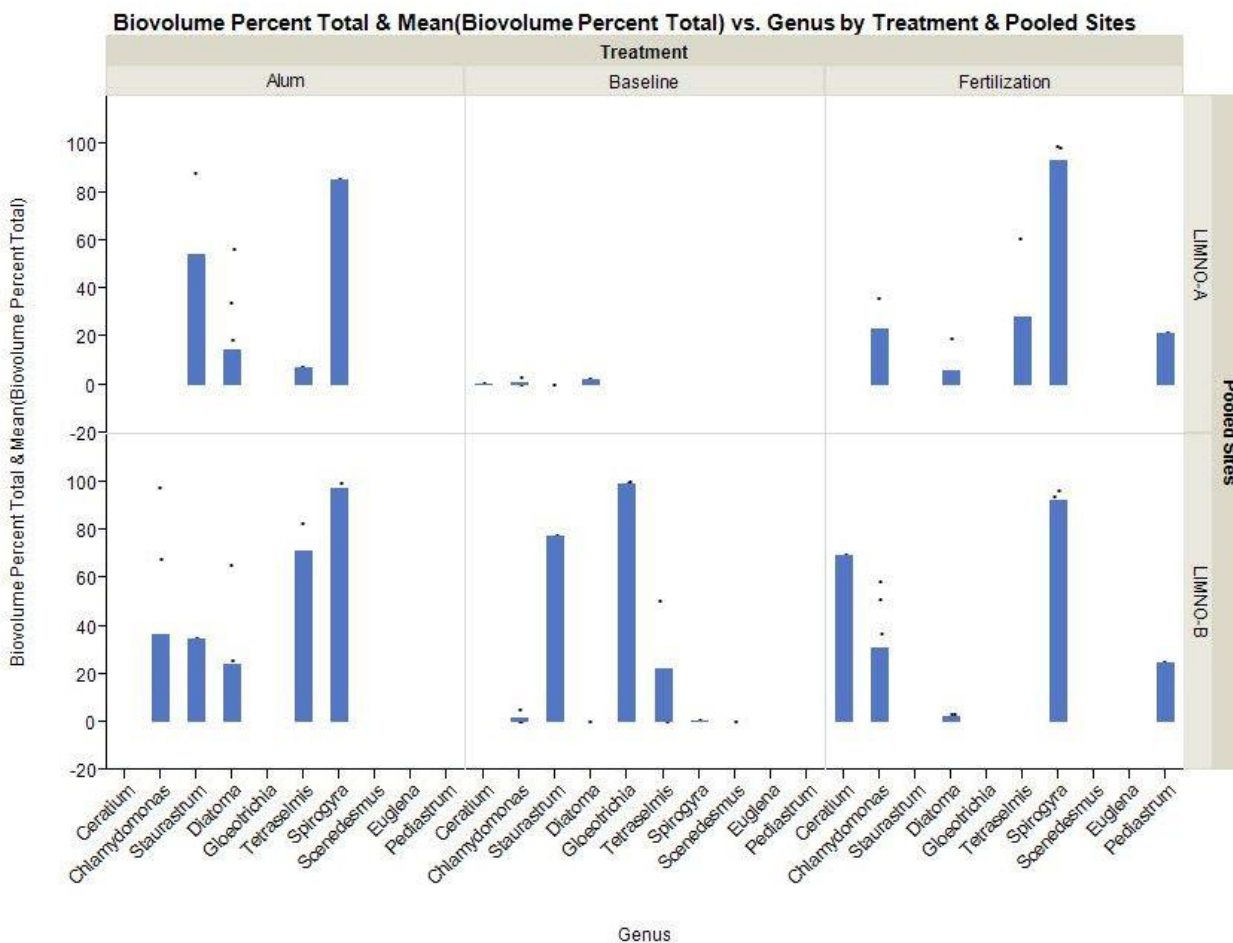
On a natural unit per mL basis, the chlorophyte *Spirogyra* occurred in greatest numbers during the fertilization treatment (Fig. 25). The flagellated chlorophyte *Chlamydomonas* and the chrysophyte *Diatoma* were frequently observed in almost all treatments and conditions. The only time the cyanobacteria *Gloeotrichia* was observed was during the baseline condition.

Figure 25. Units/mL vs. Genus by Treatment and Pooled Sites



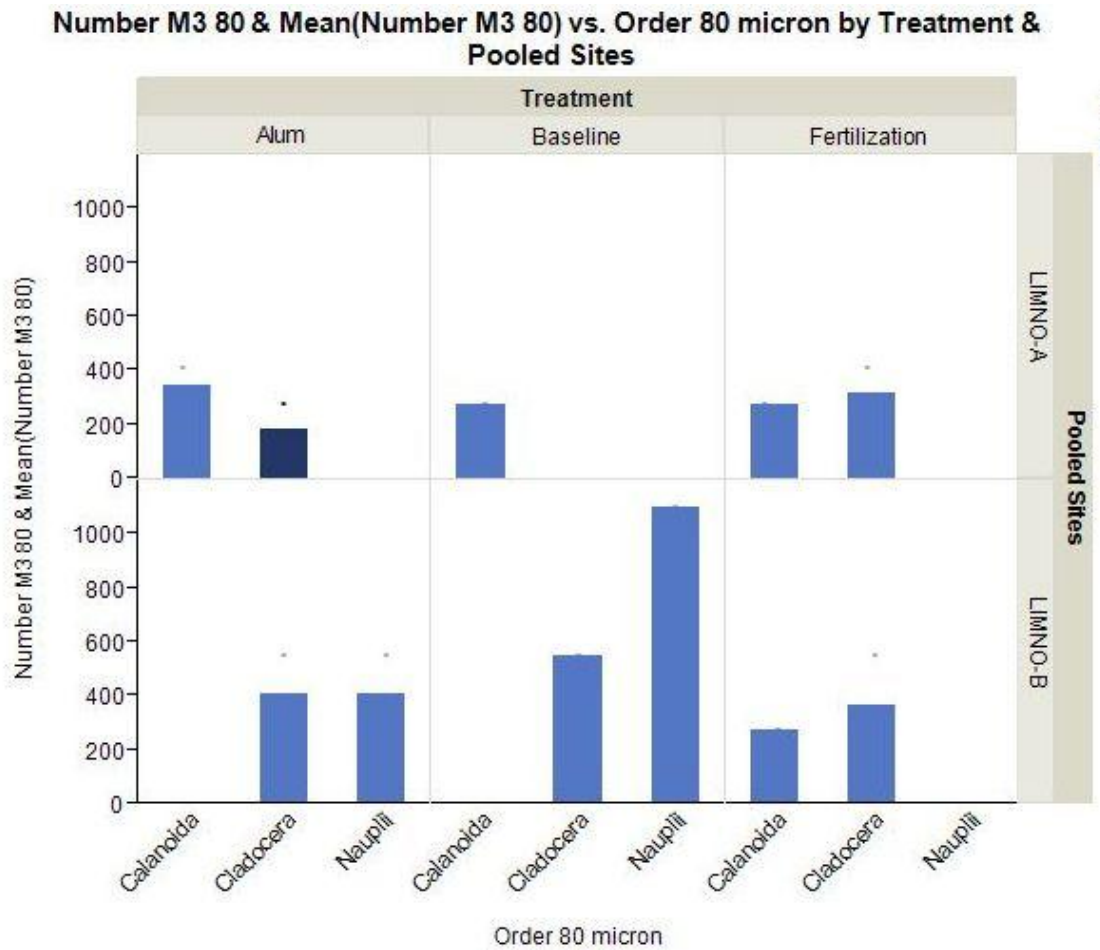
Biovolume, as measured as a percent of the total biovolume for any given sample, revealed that, when present, *Gloeotrichia* dominated as the largest species followed closely by the chlorophyte *Spirogyra* (Fig. 26). The alum and fertilization treatments were when *Spirogyra* dominated as the species comprising the largest biovolume.

Figure 26. Biovolume Percent Total vs. Genus by Treatment and Pooled Sites



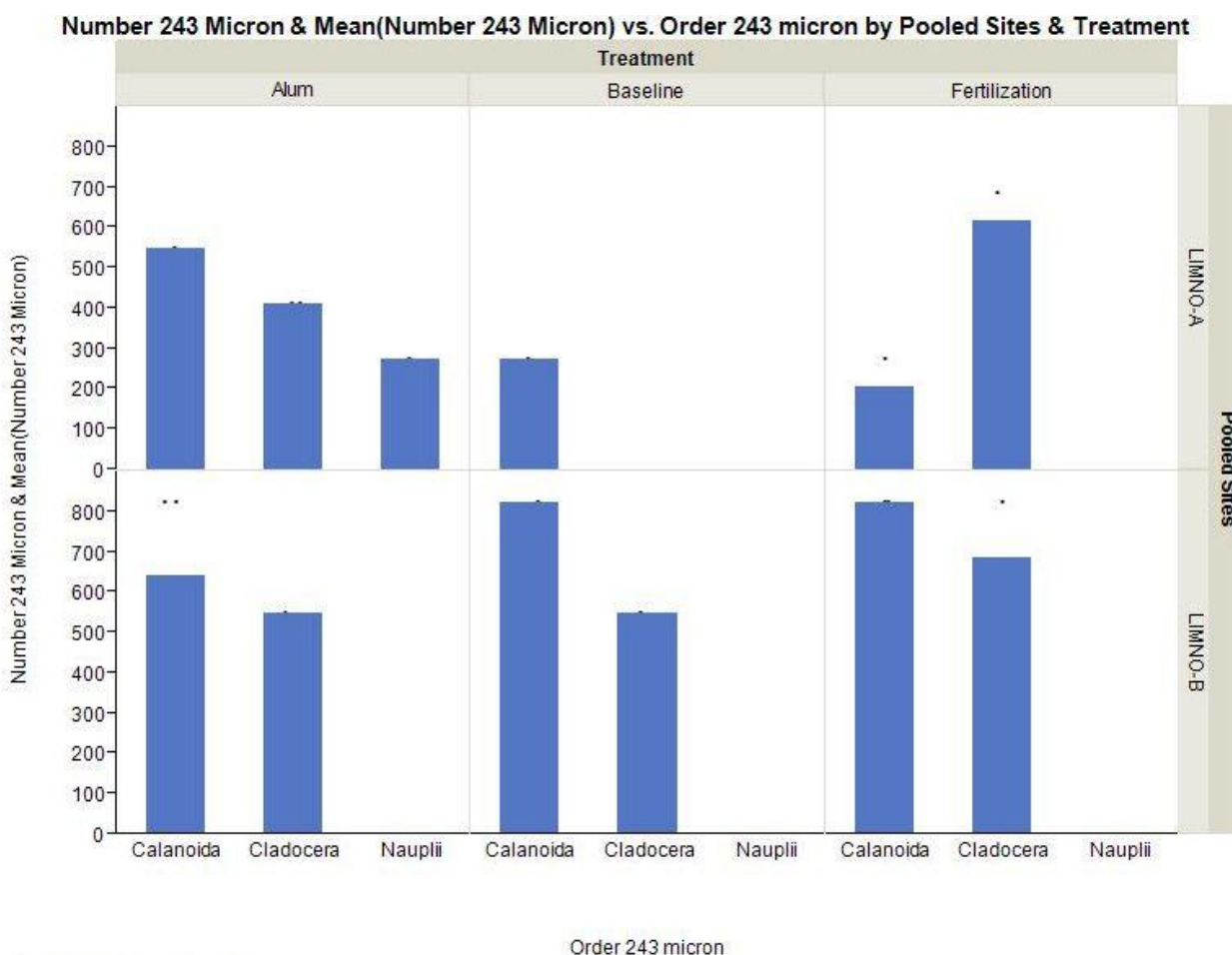
Two net mesh sizes were used for collection of zooplankton, 80 and 243 μm . For the 80 μm mesh size, copepod nauplii were found in the highest numbers during the baseline condition followed by the baseline condition (Fig. 27). None were found during the fertilization treatment. Adult calanoid copepods were found more often at site Limno-A and cladocerans (likely of the Genus *Daphnia*) were more commonly found at site Limno-B.

Figure 27. Number M³ vs. Order by Treatment and Pooled Sites



The only time nauplii were found in the 243 μm mesh size was during the alum treatment at site Limno-A (Fig. 28). Interestingly, calanoid copepods now dominated the zooplankton assemblage at Limno-B as compared to the 80 μm mesh size when these were largely absent. Also interesting is that the alum and fertilization treatments appeared to have more total zooplankters than did the baseline condition at Limno-A.

Figure 28. Number M³ vs. Order by Treatment and Pooled Sites



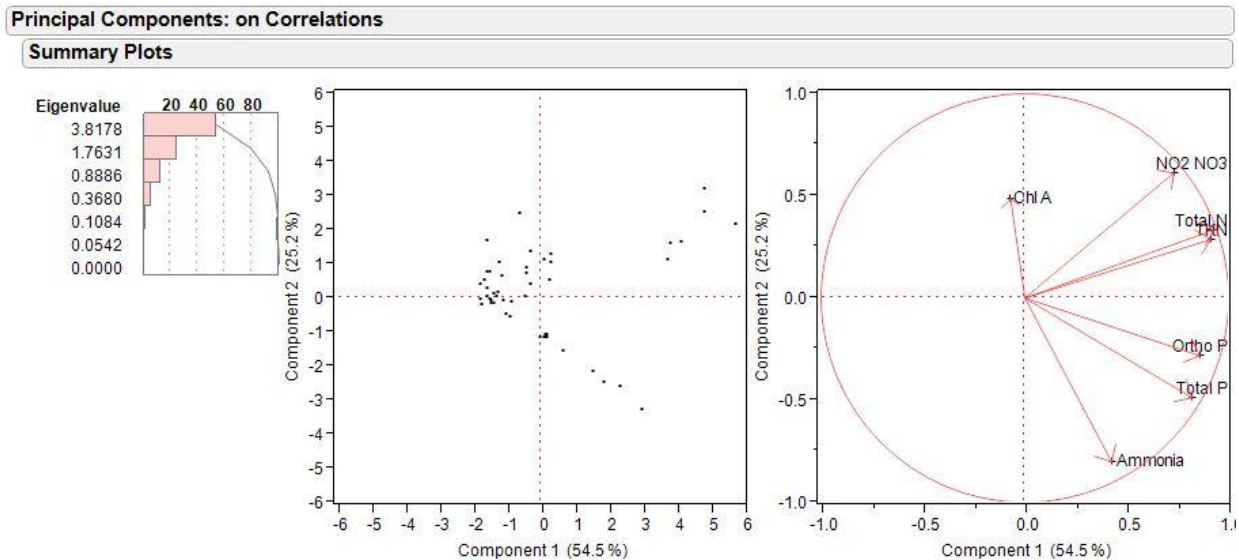
Relationships

We used principal components analysis to examine the relationships between and among many variables in the high dimensional data set. Of primary importance was what variables were the most important in causing primary production in the limno-corrals. Because there are different measures of primary production (including standing crop), and because the correlations between these different measures are nebulous, we will analyze algal nutrients against measures of chlorophyll a, counts (units/mL), and biovolume.

Measures of chlorophyll a had one of the more nebulous relationships to algal nutrients (Fig. 29). Chlorophyll a was most closely correlated to measures of organic and oxidized forms

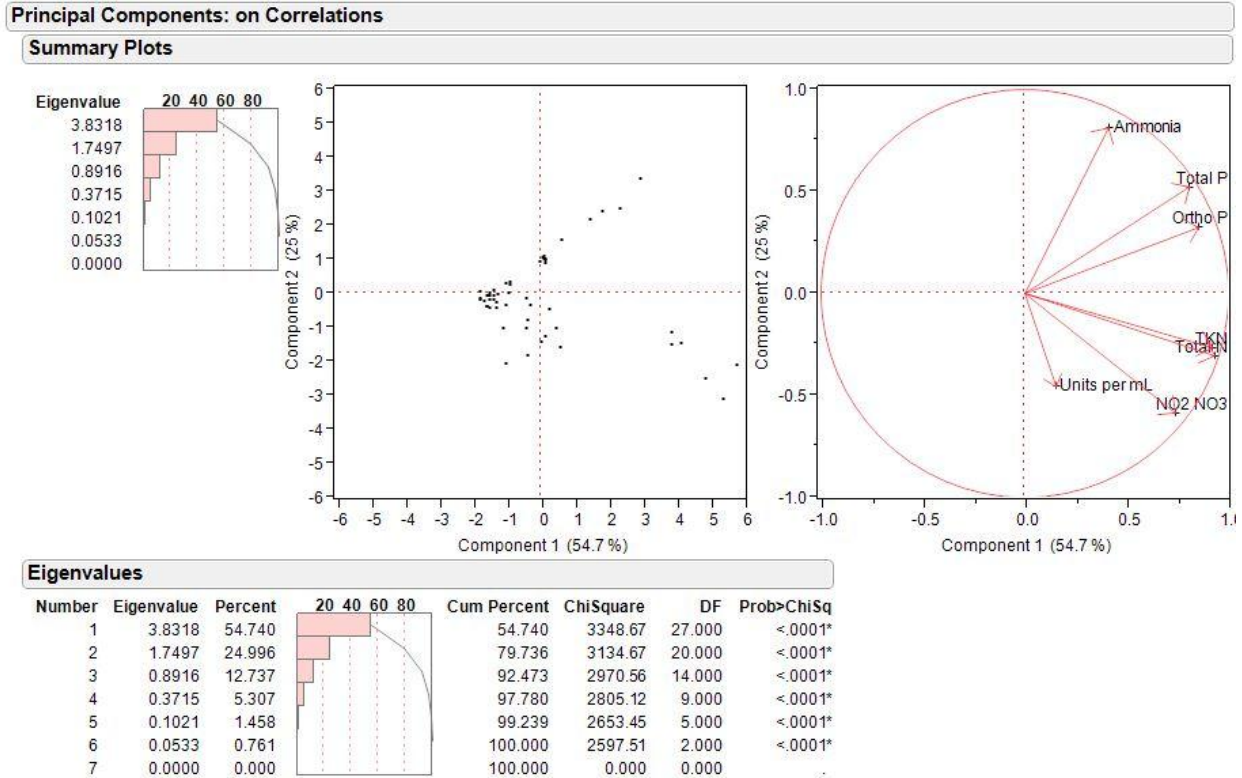
of nitrogen and seemingly an inverse correlation to reduced forms of nitrogen (Fig. 29). Care should be taken about auto-correlation between species of nitrogen as reduced forms are often found within a stratified hypolimnion outside of the photic range of most types of algae. The correlation between chlorophyll a and either TKN or total N are very similar. Therefore, it is likely safe to assume that there is at least some correlation between chlorophyll a and at least total nitrogen.

Figure 29. Principal Components Analysis of Chlorophyll a and Algal Nutrients.



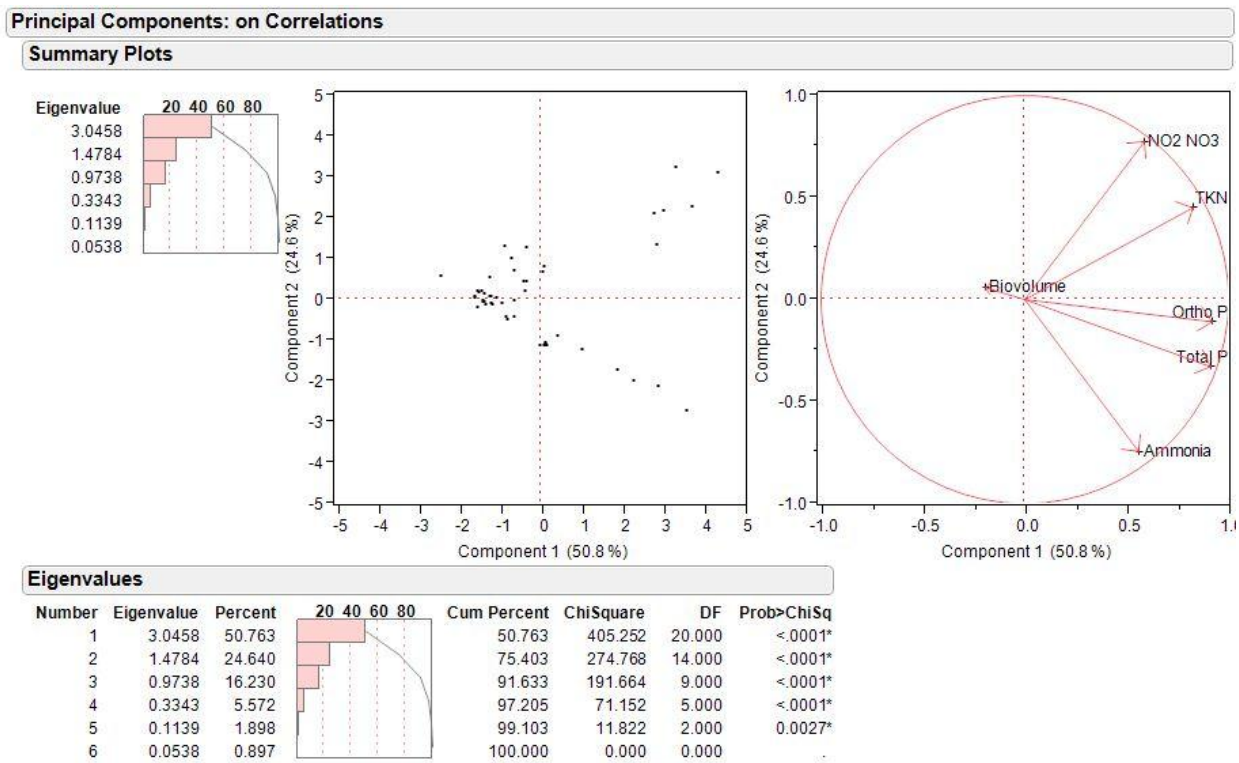
Algae count data (in units/mL) showed a similar relationship to algal nutrients as chlorophyll a (Fig. 30). There was a positive relationship to all forms of nitrogen with the exception of ammonia.

Figure 30. Principal Components Analysis of Units/mL and Algal Nutrients.



Interestingly, measures of biovolume showed an inverse relationship to virtually all algal nutrients (Fig. 31). This actually makes sense due to the cyanobacter *Gloeotrichia* dominating the algal assemblage biovolumetrically during the baseline condition. The divisional shift away from cyanobacteria to, for the most part, smaller-bodied chlorophytes during the fertilization treatment is likely the main reason for the inverse relationship between biovolume measurements and algal nutrients.

Figure 31. Principal Components Analysis of Biovolume and Algal Nutrients.



DISCUSSION

The close reproducibility between replicates, response to algal nutrient levels following treatment(s), and similarity to lake conditions during baseline conditions were all very successful components of this experiment. Less successful was the algal response to fertilization and alum treatments. This is likely due to several factors. Algal assemblages are highly dynamic and temporal variability is very difficult to capture in field or even laboratory studies. This project was started late in the growing season for a lake at the elevation of Watson and time given for the algal response to each treatment likely far too short. Also, the limno-corrals have a light transparency of ~ 85% ambient light. The amount of periphytic biomass growing on the inside of the limno-corrals was not expected and was not accounted for in this experiment. This likely resulted in under-estimating the amount of algal biomass inside the limno-corrals and affected the results; especially during the fertilization treatment. Had this biomass been accounted for, it's possible the measures of algal biomass would have been more significant between treatments and baseline condition.

The cyanobacter *Gloeotrichia* is difficult to sample representatively. It was observed in Watson as macroscopic balls suspended at varying depths throughout the water column. It was also observed *in situ* in each limno-corral during the baseline condition. In fact each spherical colony or “ball” contains many pseudo-filaments adjoined together (Picture X). *Gloeotrichia* is usually found to contain heterocysts for nitrogen fixation. Each spherical colony is joined together at the heterocystous end of each pseudo-filament. Each colony is very large and on a biovolumetric basis, is often dominate in the water column. Within the lake it appeared as if *Gloeotrichia* was the dominant phytoplankter with little growing in the space between each colony. Despite this, it was found in relatively low levels in grab or composite samples collected from the lake (personal observation). This is likely due to a failure to adequately and representatively capture such large phytoplankton using the gear selected.

The presence and apparent dominance of *Gloeotrichia* both in the lake and limno-corrals during the baseline condition is telling. As previously mentioned, this is a highly heterocystous species capable of fixing atmospheric nitrogen. Given the total biovolume observed *in situ* within the lake, but not necessarily reflected in collected samples, we could assume there is a relatively large degree of nitrogen fixation occurring within the lake. The presence or absence, or speciation, of nitrogen within the lake and the presence of an abundance of species capable of N_2 fixation leads to the potential for a few scenarios. Before N_2 can be incorporated into biological molecules, it must be converted to NH_3 . The biological reduction of N_2 is catalyzed by a multimeric enzyme complex, nitrogenase. This enzyme is irreversibly inhibited by molecular oxygen. The specialized heterocystous cells where nitrogen fixation occurs, walls off oxygen NH_3 from surrounding cells. The presence of such a highly heterocystous species such as *Gloeotrichia* indicates the possibility of nitrogen limitation in the surrounding water, giving it a decisive advantage over other phytoplanktonic species.

Upon the introduction of nitrogen and phosphorous during the fertilization treatment, the advantage *Gloeotrichia* had over other species was removed. This resulted in smaller-bodied algal cells such as flagellated chlorophytes becoming dominant. This new assemblage requires not only light but also oxidized forms of nitrogen such as NO_3 and to a much smaller degree,

NO₂. The new nutrient ratios and levels favored an abrupt and total assemblage shift in a relatively very small period of time. This assemblage shift not only occurred in the phytoplankton, but also in the amount of periphytic biomass which was readily observed growing inside the limno-corrals. This sudden growth (primarily of filamentous *Spirogyra*) and overall biomass of periphyton was un-expected given the relatively short duration of the experiment. Unfortunately, this periphytic biomass would have been difficult to accurately quantify and was not. However, personal observation revealed that it was largely absent almost immediately following alum treatments likely due to nutrient limitation of P. Had this periphytic biomass been quantified, results of chlorophyll *a* and algal counts likely would have been more significant.

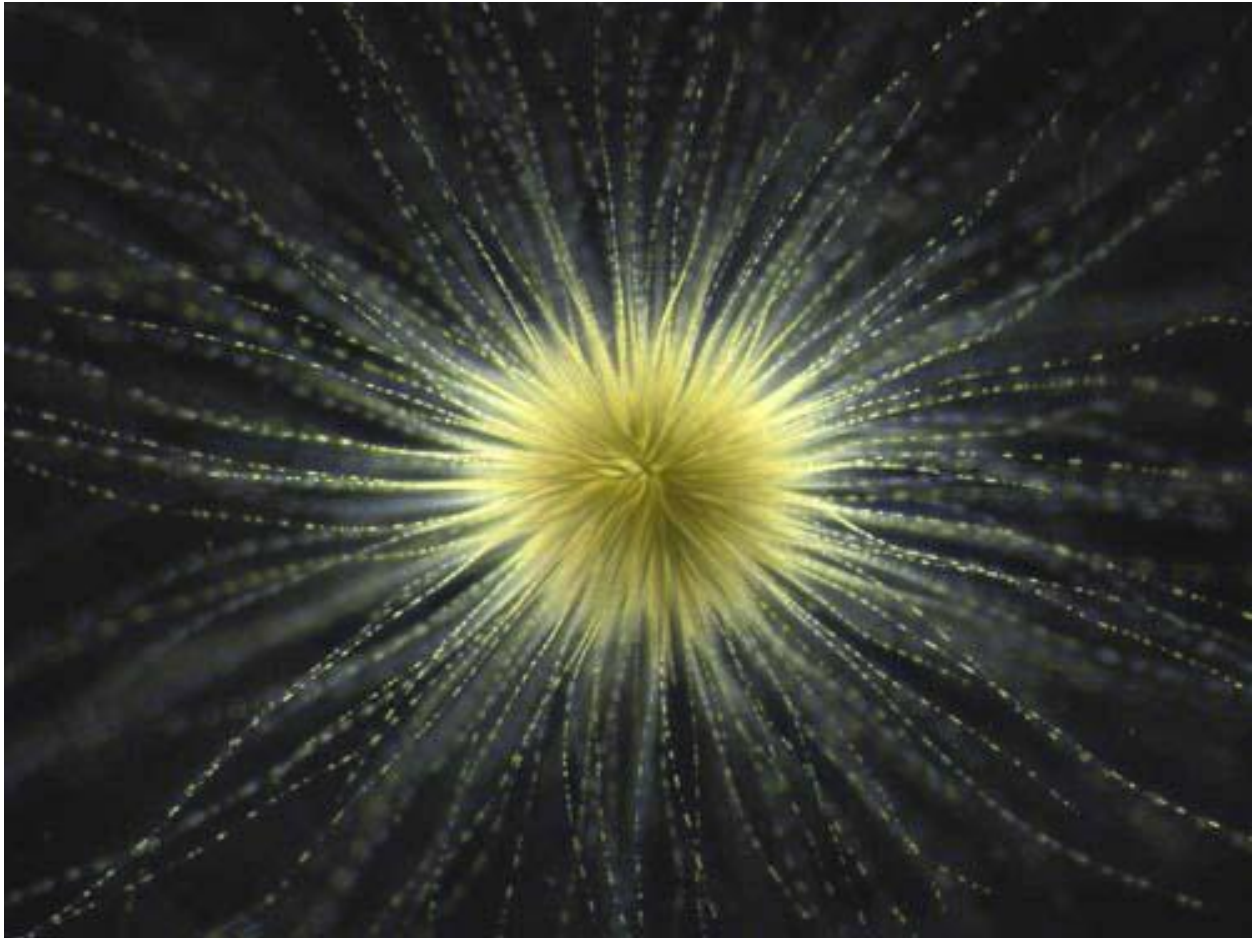
Ammonification occurred in the hypolimnia of Watson. Certainly some of this ammonification occurred from organic matter produced in the photic zone raining down to bottom, anoxic waters. Some of it, however, likely comes from reduction within lake sediments and the release of ammonia from them. Due to the inter-conversion of nitrogen species (oxidized, organic, or oxidized), the overall nitrogen pool within Watson seems to favor species capable of nitrogen fixation. The hypolimnion may essentially “lock up” nitrogen in the form of ammonia as Summer progresses and surface waters deposit organic nitrogen to bottom waters. There may be more algal diversity in the photic zone earlier in Summer or Spring. It would be interesting to try an aeration-type experiment within the limno-corrals to determine if aeration could play a significant role in reducing nutrient (N and P) within the lake.

There was no significant difference in overall zooplankton levels between the baseline and alum treatments. Herbivorous zooplankters always lag behind any increase or decrease in phytoplankton numbers and biomass. Zooplankton did seem to increase during the fertilization treatment, however, these results might have been even more significant had time allowed us to follow trends in zooplankton numbers over time. There appeared to be little or no toxicity concerns following alum treatment.

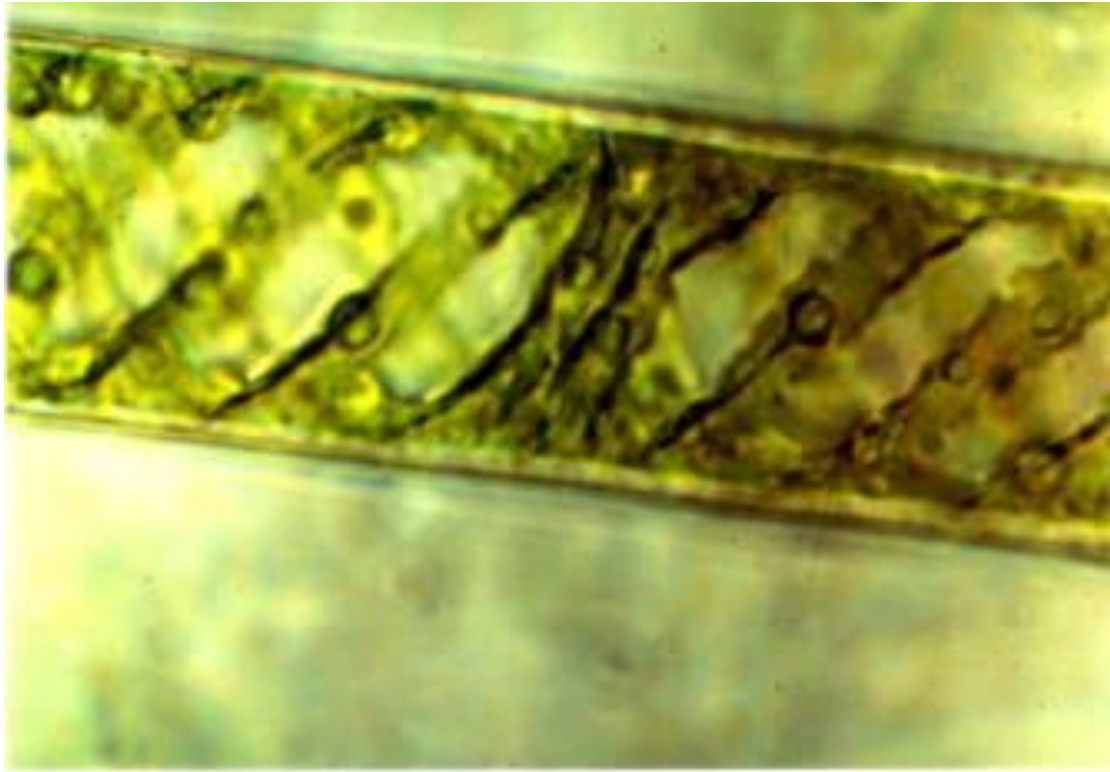
The effects of alum on the baseline condition are unknown. It also would have been interesting to see if P-limitation would have resulted in another algal assemblage or reduction in biomass as compared to what was already in the lake. Time and budgetary constraints kept us from performing such an analysis in this experiment. In any future limno-coral experiment, the effect(s) of treatments on the baseline condition should be established.

The effect of fertilization on the baseline treatment resulted in a changed algal assemblage. This new assemblage did not favor species capable of N₂ fixation. Algal biomass within the limno-corrals likely changed more than was noticed had periphyton been quantified. What is clear is that algal assemblages react quickly to nutrient addition...and nutrient removal. The dynamics of this could be observed in this study and were successful in regards to nutrient addition and removal. The effects this had on the algal assemblage leads insight into what might occur in Watson under the scenarios of either increasing or decreasing nutrient concentrations. For any subsequent use of limno-corrals, more time is needed to capture temporal variability and periphyton needs to be quantified.

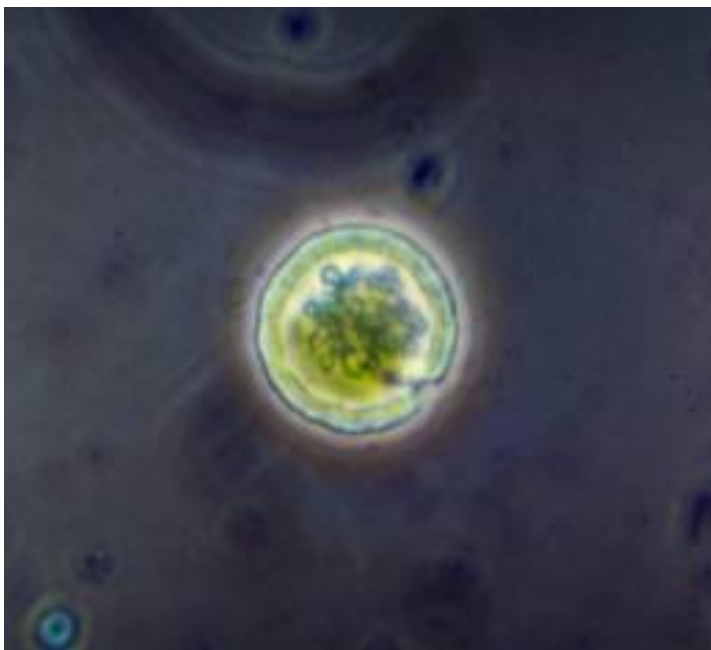
PICTURE 1. *Gloecystis* sp. found within Watson Lake limno-corrals 100 X



PICTURE 2. *Spirogyra* sp. found within Watson Lake limno-corrals. 150 X



PICTURE 3. *Chlamydomonas* sp. found within Watson Lake limno-corrals. 200 X



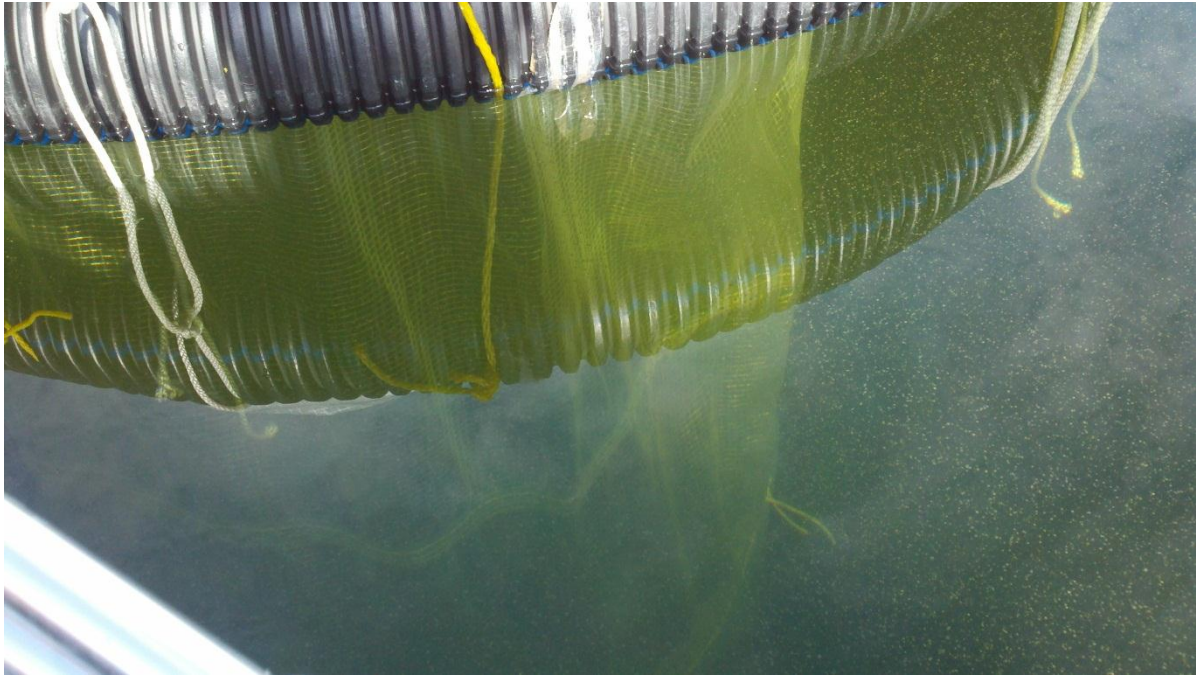
PICTURE 4. Limno-corrals loaded on pontoon boat for deployment.



PICTURE 5. Limno-corrals deployed within Watson Lake.



PICTURE 6. Outside of limno-corrals.



PICTURE 7. Collecting zooplankton from limno-corrals.

